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Evaluation of BBL CHROMagar VanRE for Detection of Vancomycin-Resistant Enterococci in Rectal Swab Specimens[▽]

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A study was performed on 517 surveillance rectal swabs to evaluate a selective and differential chromogenic medium, the BBL CHROMagar VanRE (CVRE), which enables recovery and identification of VanA- and VanB-containing *Enterococcus faecium* (ENFM) and *Enterococcus faecalis* (ENFS) isolates. Compared to BBL Enterococcosel agar, a bile-esculin-azide-vancomycin (BEAV) agar, the initial overall sensitivity, specificity, and positive and negative predictive values of CVRE for the detection of vancomycin-resistant ENFM and ENFS were 99.1% and 94.8% and 84.2% and 99.7%, respectively. Among our patient population, more vancomycin-resistant enterococci (VRE) were recovered with CVRE than BEAV.

Vancomycin-resistant enterococci (VRE) are major causes of nosocomial infections in health care facilities, and those patients infected with VRE have worse outcomes while hospitalized (8). Rapid, reliable identification of these antibiotic-resistant organisms is crucial for patient management and infection control measures (9, 12).

Culture from rectal swabs or stool specimens onto bile-esculin-azide agar with vancomycin (BEAV) is the VRE screening method used in many clinical laboratories. Confirmation of VRE using this medium requires 48 to 72 h. Chromogenic agars to detect VRE demonstrate promise (1-7, 10). BBL CHROMagar VanRE (CVRE; BD Diagnostics, Sparks, MD) is a selective and differential chromogenic agar under development for the detection of vancomycin-resistant *E. faecium* (VRENFM) and vancomycin-resistant *Enterococcus faecalis* (VRENFS). CVRE contains 8 μg/ml of vancomycin and uses chromogenic substrates to phenotypically differentiate VRENFM as mauve colonies and VRENFS as green colonies. Other bacteria are inhibited or typically grow as a color other than mauve or green. Our study compared the clinical performance of CVRE with that of BEAV for primary isolation and detection of VRE from surveillance rectal swabs.

Patient samples. This industry-sponsored clinical trial was approved by the Johns Hopkins University School of Medicine Institutional Review Board. At the Johns Hopkins Hospital, VRE surveillance cultures are obtained weekly from patients in all intensive care units (ICUs) and from other high-risk groups, such as oncology, transplant, and HIV patients. Multiple specimens per patient were permitted in the study if previous cultures were negative. Two positive specimens were admissible, provided the specimens were collected >5 days apart.

Surveillance rectal swabs were first inoculated onto BEAV followed by inoculation onto CVRE. Both plates were aseptically streaked for isolation and incubated at 37°C for 24 to 48 h.

BEAV. BEAV plates with 6 μg/ml of vancomycin were incubated aerobically. No-growth cultures or those not consistent with VRE by 48 h had no further workup. Presumptive colonies for VRE (black colonies with a Gram stain of positive cocci) were isolated to 5% sheep blood agar (SBA) and incubated for an additional 18 to 24 h. L-Pyrrolidonyl-β-naphthylamide enzyme (PYR)-positive colonies were identified with the BD Phoenix automated microbiology system (BD Diagnostics, Sparks, MD). Vancomycin susceptibility testing was performed by using broth microdilution panels that were manufactured and quality tested by the sponsor. VRENFS ATCC 51299 and *E. faecalis* ATCC 29212 were used as controls on each day of susceptibility testing. Vancomycin-susceptible cultures (MIC, <8 μg/ml) and/or isolates that were not identified as *E. faecium* or *E. faecalis* were determined to be negative for VRE. Cultures containing *E. faecium* and/or *E. faecalis* with a vancomycin MIC of ≥8 μg/ml were considered positive for VRE.

CVRE. Positive controls, VRENFM ATCC 700221 (mauve) and VRENFS ATCC 51299 (green), and negative controls, *Escherichia coli* ATCC 25922 (no growth) and *E. faecalis* ATCC 29212 (no growth), were used on each day of testing. The plates were placed in a vented box to maintain a 5% CO_2 atmosphere while being protected from light. Cultures exhibiting no growth by 48 h or colonies not consistent with VRE (not mauve or green colonies) had no further workup. Mauve colonies (*E. faecium*) or green colonies (*E. faecalis*) that were Gram-positive cocci and catalase negative were considered positive for VRE at either 24 or 48 h.

Data analysis. Using Stata 9.2 (StataCorp LP, College Station, TX), we initially compared the performance of CVRE to that of BEAV (reference method). Catalase-negative, Gram-positive cocci that grew as mauve or green isolates from the CVRE agar were compared to the identification of the enterococci recovered from the BEAV agar. A second performance

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TABLE 1. Initial recovery of *E. faecium* (mauve) and *E. faecalis* (green) from CVRE cultures compared to identification from BEAV cultures at 48 h[a]

CVRE result[b]	No. of cultures with indicated BEAV result			% sensitivity (95% CI)	% specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
	Positive	Negative	Total				
Vancomycin-resistant *E. faecium*							
Mauve colony (GPC) and catalase negative	97	17	114[c]	99.0 (94.5–100)	95.9 (93.6–97.6)	85.1 (77.2–91.1)	99.8 (98.6–100)
Negative	1	402	403				
Total	98	419	517				
Vancomycin-resistant *E. faecalis*							
Green colony (GPC) and catalase negative	17	18	35	100 (80.5–100)	96.4 (94.4–97.9)	48.6 (31.4–66.0)	100 (99.2–100)
Negative	0	482	482				
Total	17	500	517				
Vancomycin-resistant *E. faecium* and/or *E. faecalis*							
Mauve and/or green (GPC) and catalase negative	112	21	133[c]	99.1 (95.2–100)	94.8 (92.2–96.8)	84.2 (76.9–90.0)	99.7 (98.6–100)
Negative	1	383	384				
Total	113	404	517				

[a] CVRE, BBL CHROMagar VanRE (BD Diagnostics, Sparks, MD); BEAV, BBL bile-esculin-azide-vancomycin agar with 6 μg/ml of vancomycin (BD Diagnostics, Sparks, MD); 95% CI, 95% confidence interval; PPV, positive predictive value; NPV, negative predictive value; GPC, Gram-positive cocci.
[b] Vacomycin resistance was defined as a MIC of ≥8 μg/ml.
[c] Eleven additional colonies were green but were catalase positive. One green *Staphylococcus* isolate was mixed with VRENFM; 10 isolates were found on individual CVRE plates (see the text and Table 2).

analysis was done which included recovery of any VRE from CVRE and BEAV.

Discrepant analysis. VRE isolated only from BEAV or CVRE-positive isolates that had no corresponding colony on BEAV were considered discrepant isolates. Identification and susceptibility testing were performed.

Findings. Over a 13-week-period, 517 rectal samples from 410 patients were screened for VRE. A total of 143 CVRE cultures presented with mauve and/or green colonies (Table 1). Seventeen of those cultures contained both colony types. The overall agreement before discrepant analysis between CVRE and BEAV was 95.7% for recovery of VRENFM and VRENFS.

Mauve colonies were recovered from 114 specimens, and all were catalase-negative, Gram-positive cocci. Of these 114 specimens, 97 VRENFM isolates were recovered on the BEAV plate; 1 VRENFM isolate was only detected on the BEAV plate and was not detected on the corresponding CVRE plate (Table 1). A total of 402 cultures were concordantly negative for VRENFM. Of the 17 mauve colonies on CVRE with no corresponding VRENFM on the BEAV plate, all were identified by the BD Phoenix as *E. faecium*. After susceptibility testing by broth microdilution, 16 were confirmed as vancomycin-resistant (MIC, ≥8 μg/ml) and were resolved as true positives (Table 2). One mauve isolate failed to grow in the broth microdilution trays. This isolate was therefore considered a vancomycin-susceptible *E. faecium* isolate (false positive). Green colonies were isolated from 35 specimens, but VRENFS was recovered from only 17 corresponding BEAV cultures (Table 1). All VRENFS isolates recovered from BEAV ($n = 17$) were recovered on CVRE. Initially, there were 482 matched samples from which no VRENFS isolates were recovered. Eighteen CVRE plates grew green isolates, but the corresponding BEAV plates were negative for VRENFS. VRENFS was confirmed on 4 of these 18 cultures with green colonies; of these 4, 3 plates had both mauve and green colonies (which resulted in VRENFM and VRENFS), and 1 showed only green colonies (which resulted in VRENFS). Of the remaining 14 CVRE/BEAV discrepant results, 11 green colonies were vancomycin-susceptible *E. faecalis*, 2 of the green colonies were identified as susceptible *Enterococcus raffinosus*, and one was vancomycin-resistant *Enterococcus galli-*

TABLE 2. Recovery of *E. faecium* and *E. faecalis* from CVRE medium 48 h after discrepant analysis of isolates from CVRE and BEAV[a]

Organism	No. of results[b]:				% sensitivity (95% CI)	% specificity (95% CI)	% PPV (95% CI)	% NPV (95% CI)
	TP	FN	TN	FP				
VRENFM	113	1	402	1	99.1 (95.2–100)	99.8 (98.6–100)	99.1 (95.2–100)	99.8 (98.6–100)
VRENFS	21	0	482	14	100 (83.9–100)	97.2 (95.3–98.5)	60.0 (42.1–76.1)	100 (99.2–100)

[a] CVRE, BBL CHROMagar VanRE (BD Diagnostics, Sparks, MD); BEAV, BBL bile-esculin-azide-vancomycin agar (BD Diagnostics, Sparks, MD); VRENFM, vancomycin-resistant *Enterococcus faecium*; VRENFS, vancomycin-resistant *Enterococcus faecalis*; 95% CI, 95% confidence interval; PPV, positive predictive value; NPV, negative predictive value.
[b] A true-positive (TP) result is defined as a mauve and/or green colony, Gram-positive coccus, and catalase negative with a vancomycin-resistant (MIC, ≥8 μg/ml) *E. faecium* or *E. faecalis* isolate obtained from BEAV or CVRE. A false-negative (FN) result is defined as VRENFM or VRENFS only isolated from BEAV. A true-negative (TN) result is defined as no mauve and/or green colonies testing as a Gram-positive coccus and catalase-negative or no vancomycin-resistant (≥8 μg/ml) *E. faecium* or *E. faecalis* isolates obtained from BEAV or CVRE. A false-positive (FP) result is defined as a mauve and/or green colony, Gram-positive coccus, and catalase negative without a vancomycin-resistant (≥8 μg/ml) *E. faecium* or *E. faecalis* isolate obtained from BEAV or CVRE.

narum (VRENGA) (MIC, 8 μg/ml). Nine VRENGA isolates were obtained from the BEAV plates. On the corresponding CVRE plates, seven had no growth and two had colonies that were not mauve or green. One isolate from the BEAV, *Enterococcus avium*, presented as the result "other color" on CVRE and failed to grow for susceptibility testing. An additional 11 isolates on the CVRE were green colonies (catalase positive) that were identified as coagulase negative *Staphylococcus* sp. On the BEAV plates, 14 breakthrough bile-esculin-positive (black) Gram-positive rods were noted.

After workup of the mauve and green isolates on the CVRE, the performance characteristics of the agar improved (Table 2). A total of 134 true-positive VRE isolates were recovered from the CVRE plates, for an overall sensitivity of detection of any VRE of 99.2%. The change in performance characteristics stratified by species improved significantly only for VREFM (specificity increased to 99.8%; $P \leq 0.05$) (Table 2).

Conclusions. Several formulations of commercially prepared chromogenic agar for detection of VRE in surveillance specimens can be found in the published literature. These are ChromID VRE agar (cIDVRE; bioMérieux, Marcy-l'Etoile, France) (1–4, 6, 7), Chromogenic VRE agar (AES VRE agar; AES Laboratoire, Bruz Cedex, France) (1), CHROMagar VRE (CHR-VRE; CHROMagar, Paris, France) (10), and BBL CHROMagar VanRE (CVRE; BD Diagnostics, Sparks, MD) (5). Ours is the second publication on the use of BBL CHROMagar VanRE with surveillance cultures, but it is the first regarding the performance of CVRE with rectal swabs.

Of the four published studies comparable to our evaluation, two tested stool specimens alone (5, 7), one study tested rectal swabs alone (2), and Grabsch et al. (4) tested both specimen types. The sensitivity of the BEAV from three studies in which BEAV sensitivity could be determined ranged from 88.2 to 93.9% (2, 4, 7).

Our evaluation is most comparable to that of Kallstrom et al. (5), who compared CVRE to BEAV with 8 μg/ml of vancomycin. The increased concentration of vancomycin in the BEAV agar used in their study did not impact primary isolation of VRE from BEAV. There was no difference in the recovery of VRENFM, other than in the study by Kallstrom et al. (5), who reported one green VRENFM colony. We experienced a larger proportion (40% for $n = 14$ versus 23% for $n = 5$; $P = 0.251$) of false-positive green colonies on the CVRE, with the majority of our isolates being either *E. gallinarum* or vancomycin-susceptible *E. faecalis*. The data from Kallstrom et al. (5) support our conclusion that the medium performs well for mauve colonies, but green colonies should be identified to the species level and have vancomycin susceptibility determined. Although there was a trend of more false-positive results with rectal swabs with the CVRE in our laboratory, as opposed to the stool specimens used by Kallstrom et al. (5), there was no statistical difference at 48 h in the recovery and detection of VRENFM ($P = 1.000$) or VRENFS ($P = 0.251$).

All studies reported similar rates of recovery of both VRENFM and VRENFS from chromogenic agars to those from BEAV (1, 2, 4-7, 10). CVRE appeared to perform better than the other chromogenic agars compared to BEAV, but a direct comparison is needed to provide the most accurate comparison. In our study, unless plates had clearly defined isolated black colonies on BEAV or clearly defined isolated mauve and/or green colonies at 24 h, plates were held until the second day before workup reliably proceeded. Approximately 70% of the positives on CVRE were discernible at 24 h. Disadvantages with BEAV include the fact that a black colony is not specific for VRE, nor can BEAV differentiate among species of enterococci. Two additional days are required to subculture a black colony for biochemical and susceptibility testing, whereas subculture is only necessary for green colonies on CVRE.

Currently cIDVRE (bioMérieux, Marcy-l'Etoile, France) is the only FDA-cleared chromogenic agar for use in clinical laboratories. Chromogenic media are expected to list at a higher cost per plate than bile-esculin-azide agars with vancomycin. cIDVRE will list for approximately $3.00 (U.S. dollars) per plate, and the CVRE assay (BD Diagnostics, Sparks, MD), currently not available, will be comparable in price to the BBL CHROMagar MRSA (methicillin-resistant *Staphylococcus aureus*) assay at $6.70 per plate. BBL Enterococcosel agar (BEAV with 6 μg/ml of vancomycin) costs $2.30 per plate. However, the cost to identify an isolate from BEAV is not limited to isolation from the plate. Additional tests are required (Gram stain, catalase, and PYR) to identify an isolate as *Enterococcus* sp. Determination of vancomycin susceptibility is also required, which greatly adds time and expense to the BEAV culture. Thus, the cost for the chromogenic agars compared to BEAV is offset by the ability to detect and isolate VRE (mauve and/or green colonies) more rapidly and easily with minimal supplemental testing.

A limitation of this study was that the sample size for VRENFS was small ($n = 21$), but the prevalence of VRENFS in this study is consistent with past work conducted at our institution (11). Similarly, we did not pursue genotypic testing for the mechanisms of vancomycin resistance as this was beyond the scope of this study. In the past, our population has presented with a low prevalence of *vanB*-mediated resistance (11).

In summary, the BBL CHROMagar VanRE (CVRE) performed well, and the chromogenic agar formulation was easy to use. We recovered an additional 20 VRE isolates (16 VRENFM and 4 VRENFS) that otherwise would have been missed by the BEAV agar. In our population, mauve colonies consistently tested as *E. faecium* (100%) and were usually vancomycin resistant (99.1%); therefore, we are comfortable reporting all mauve, catalase-negative, Gram-positive cocci as VRENFM. Although all VRENFS isolates were detected on the CVRE medium, 40% (14/35) of the green, catalase-negative, Gram-positive isolates in our population were not identified as VRENFS. Based on our data, green colonies from the CVRE should be identified and susceptibility tests should be performed. Use of chromogenic agar in VRE surveillance should facilitate infection control practices by identifying VRE carriers more easily, sooner, and without a substantial increase in laboratory costs.

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